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Year in School: Senior
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Funding Source: Life Sciences Undergraduate Research Opportunity Program

Using H1G1.1c3 cells to determine TCDD-EQs for environmental samples

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that is activated by the binding of halogenated and polycyclic aromatic hydrocarbons (HAHs and PAHs). The 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent HAH, which has the ability to biomagnify in the food chain and has adverse effects in humans and animals. The current EROD (Ethoxyresorufin-O-Deethylase) H4IIE bioassay is routinely used to develop TCDD equivalency factors (TEQs) in environmental samples. The assay indirectly measures EROD produced due to the presence of PAH-like compounds which bind to the AhR receptor, inducing cytochrome P4501A (CYP1A) and transcribing production of EROD. Through the addition of ethoxyresorufin, EROD produces a fluorescent resorufin product, which is measured and compared to the induction of CYP1A by TCDD to give a TEQ factor of the environmental sample. The current assay uses H4IIE cells, which are dosed with 7-fold serial dilutions of each environmental sample and the resulting fluorescence is measured after 72 hours. However, a new cell line (H1G1.1c3) has a green fluorescent protein (GFP) transfected into the cells to produce GFP in response to AhR binding by PAHs. Replacing the H4IIE cells in the bioassay with H1G1.1c3 cells would allow GFP to be measured directly in living cells, whereas the H4IIE cells must be lysed prior to measuring fluorescence in order to free the EROD enzyme, which is then measured indirectly with the addition of ethoxyresorufin. This gives H1G1.1c3 cells an advantage over the H4IIE strain because the GFP can be measured directly over time and requires only a single dose of each environmental extract. Using the H1G1.1c3 strain, the cells were dosed once and examined on a time-course analysis at 4, 8, 12, 48, 72, 96, and 120 hours after dosing. The potency estimates from the time-course analyses were compared with the potency estimates from the traditional dose-response analyses. The time related effective time (ET50) value was compared to the effective concentration (EC50) value for maximum induction using the H1G1.1c3 cells. The relative potency of both lines (H1G1.1c3 and H4IIE) was compared. The conclusion that H1G1.1c3 cells provide the same relative potency and maximum induction values as compared to H4IIE cells, gives the H1G1.1c3 bioassay the advantages of faster data collection and lower reagent costs than the traditional H4IIE bioassay.